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The stiff-man syndrome

MICHAEL P. PENDER

Introduction

The stiff-man syndrome is a rare disorder of the central nervous system (CNS) characterized by progressive fluctuating rigidity and painful spasms of the body musculature. It was first described in 1956 by Moersch & Woltman, although they observed the first case of this condition much earlier, in 1924. Ironically, they nicknamed the disorder 'stiff-man syndrome' to 'associate it with a memorable and descriptive term that could not be taken by anyone to be final'. Recently, evidence has accumulated that the stiff-man syndrome is an autoimmune disease directed against neurones secreting the inhibitory neurotransmitter, gamma-aminobutyric acid (GABA) (Solimena *et al.*, 1990). The syndrome usually develops spontaneously and often occurs in association with other autoimmune diseases, particularly type I (insulin-dependent) diabetes mellitus. However, it may also occur as a paraneoplastic syndrome complicating remote malignancy.

Clinical features

General clinical features and diagnosis

The characteristic clinical picture is the insidious development of muscular tightness, stiffness and rigidity, initially involving the axial musculature (neck, paraspinal and abdominal muscles) and later spreading to affect proximal limb muscles (Moersch & Woltman, 1956; Gordon, Januszko & Kaufman, 1967; Lorish, Thorsteinsson & Howard, 1989). Mobility is restricted by the simultaneous contraction of agonist and antagonist muscles, so that the patient may be observed to walk or fall like 'a wooden man'. Paraspinal rigidity may result in low-back discomfort and a prominent lordosis, and involvement of the thoracic musculature may lead to exertional dyspnoea. The cranial muscles may also be affected, with resultant difficulty in smiling, swallowing and phonating (Gordon *et al.*, 1967).

Superimposed upon the persistent muscular rigidity there are painful muscle spasms, which may be precipitated by noise, a sudden jar, voluntary movement, passive stretching of the muscles, and occasionally by fear or apprehension (Moersch & Woltman, 1956; Gordon *et al.*, 1967; Lorish *et al.*, 1989). These spasms last for several minutes. Neurological examination may reveal the muscular rigidity and spasms and resultant restriction of mobility but the motor examination is otherwise normal. The deep tendon reflexes may be increased, but the plantar responses are flexor (Lorish *et al.*, 1989). Prior to the availability of effective treatment, many patients eventually became severely disabled and totally bedbound (Moersch & Woltman, 1956; Lorish *et al.*, 1989). Paroxysmal autonomic dysfunction leading to hyperpyrexia, diaphoresis, tachypnoea, tachycardia, pupillary dilatation, arterial hypertension and sudden unexpected death may complicate the clinical picture (Mitsumoto *et al.*, 1991).

Accurate clinical diagnosis of the stiff-man syndrome is important both for patient management (see below) and for research studies. Laboratory investigations are helpful in establishing the diagnosis. Electromyography reveals continuous motor unit activity 'at rest' without other abnormalities (Lorish *et al.*, 1989). In addition to routine electromyography, simultaneous video-electroencephalographic-surface electromyographic recordings may be useful in confirming the diagnosis (Armon *et al.*, 1990). Cerebrospinal fluid (CSF) examination may reveal a normal cell count or a pleocytosis and, in some patients, oligoclonal immunoglobulin G (IgG) bands which are not present in the serum (Solimena *et al.*, 1988, 1990; Folli *et al.*, 1993; Meinck *et al.*, 1994).

Association with epilepsy

Epilepsy occurs in about 10% of patients with the stiff-man syndrome (Martinelli *et al.*, 1978; Solimena *et al.*, 1990). As this percentage is considerably higher than the prevalence of epilepsy in the general population, the association is unlikely to be coincidental. Solimena *et al.* observed that epilepsy occurred only in patients with antibodies against GABA-ergic neurones (see below). As a defect in GABA-ergic neurotransmission has been implicated in the pathophysiology of epilepsy, it is possible that the epilepsy associated with the stiff-man syndrome may also have an autoimmune basis.

Association with other autoimmune diseases

Patients with the stiff-man syndrome have an increased incidence of organ-specific autoimmune diseases, particularly insulin-dependent diabetes mellitus, but also Graves' disease, hypothyroidism, pernicious anaemia and

vitiligo (Solimena *et al.*, 1990; Grimaldi *et al.*, 1993). They also have a high incidence of organ-specific autoantibodies, namely those directed against islet cells, gastric parietal cells, thyroid microsomal fraction and thyroglobulin. The concurrence with other autoimmune diseases is seen in patients with autoantibodies against GABA-ergic neurones (see below), but not in those without such antibodies (Solimena *et al.*, 1990; Grimaldi *et al.*, 1993). This association supports the hypothesis that the stiff-man syndrome is also an autoimmune disease.

Association with malignancy

Occasionally the stiff-man syndrome occurs as a paraneoplastic syndrome complicating remote malignancy, such as breast carcinoma (Folli *et al.*, 1993), pharyngeal carcinoma (Masson *et al.*, 1987), colonic carcinoma (Piccolo & Cosi, 1989), small cell lung cancer (Bateman, Weller & Kennedy, 1990) and Hodgkin's disease (Ferrari *et al.*, 1990). It has also been observed in association with paraneoplastic limbic encephalitis (Masson *et al.*, 1987). The onset of the stiff-man syndrome may precede the detection of the associated malignancy.

Genetics

Class II HLA genes

The proportion of patients with the stiff-man syndrome who carry the HLA-DQB1*0201 allele (72%) is significantly higher than the proportion of controls who carry this allele (38%) (Pugliese *et al.*, 1993). This indicates that the stiff-man syndrome is associated with this allele, as are insulin-dependent diabetes mellitus and other autoimmune diseases. Interestingly, the diabetes-protective DQB1*0602 allele and other sequence-related DQB1*06 alleles, which are rarely found in insulin-dependent diabetes, occur with the same frequency as in controls. Diabetes is more frequent in patients with the stiff-man syndrome who lack a DQB1*06 allele than in those with such an allele, suggesting that the presence of the DQB1*0602 allele or other DQB1*06 alleles may protect against diabetes in patients with the stiff-man syndrome (Pugliese *et al.*, 1993).

Neuropathology

Perivascular lymphocytic accumulation has been observed in the spinal cord, brainstem and basal ganglia of patients with the stiff-man syndrome

with or without associated malignancy (Masson *et al.*, 1987; Bateman *et al.*, 1990; Mitsumoto *et al.*, 1991; Meinck *et al.*, 1994).

Pathophysiology

In a detailed neurophysiological study on a patient with the stiff-man syndrome, Meinck, Ricker & Conrad (1984) found abnormal enhancement of exteroceptive reflexes, particularly those elicited from the skin, but no abnormalities of the monosynaptic reflex arc. Administration of clomipramine, which results in an excess of serotonin and noradrenaline at synapses, severely aggravated the clinical symptoms. In contrast, clonidine, which leads to an inhibition of noradrenaline release, and diazepam, which increases GABA-ergic activity, decreased both the muscular stiffness and abnormal exteroceptive reflexes. Meinck *et al.* proposed that the clinical manifestations are due to a disorder of descending brainstem pathways that exert a net inhibitory control on axial and limb girdle muscle tone as well as on exteroceptive reflex transmission.

Immunological findings in the peripheral blood

Antibodies against glutamic acid decarboxylase

In 1988, Solimena *et al.* reported that the serum and the CSF of a patient with the stiff-man syndrome, epilepsy and insulin-dependent diabetes mellitus intensely and specifically stained all grey-matter regions in frozen sections of the rat brain studied by light-microscopic immunocytochemistry. The staining pattern consisted primarily of small puncta that often outlined the profiles of perikarya and dendrites, suggesting a predominant localization of immunoreactivity in a major subpopulation of synapses, each of which would be represented by a punctum. Furthermore, in all brain regions the pattern of immunoreactivity corresponded with the distribution of GABA-ergic nerve terminals and with the staining pattern obtained with antibodies to glutamic acid decarboxylase (GAD), the enzyme responsible for the synthesis of GABA. Interestingly, the serum and the CSF of this patient also intensely and specifically stained pancreatic islet beta cells, which contain a high concentration of GAD and which are destroyed in insulin-dependent diabetes mellitus. Double immunofluorescence studies revealed that this staining was almost indistinguishable from that produced by antibodies against GAD. Using Western blotting, Solimena *et al.* demonstrated that the serum and CSF labelled a band (approximately 60 kDa) with an electrophoretic mobility corresponding to that of the band

labelled by GAD antiserum. On the basis of these exciting observations they hypothesized that the stiff-man syndrome is an autoimmune disease directed against GABA-ergic neurones.

In a subsequent study, Solimena *et al.* (1990) found that 60% of patients with the stiff-man syndrome had serum antibodies against GABA-ergic neurones, with GAD being the principal autoantigen. Antibodies against GABA-ergic neurones were not found in patients with other neurological disorders. Solimena *et al.* observed that insulin-dependent diabetes mellitus occurred frequently in the patients with the stiff-man syndrome and anti-GAD antibodies. This observation led to the finding that the 64-kDa pancreatic islet beta cell antigen, which is a major target of autoantibodies in insulin-dependent diabetes, is GAD (Baekkeskov *et al.*, 1990). However, differences were observed between the anti-GAD antibodies associated with the stiff-man syndrome and those associated with insulin-dependent diabetes mellitus. The antibody titre is much higher in patients with the stiff-man syndrome. Furthermore, the anti-GAD antibodies of most patients with the stiff-man syndrome react with GAD in Western blots, whereas the anti-GAD antibodies of the majority of diabetic patients do not (Baekkeskov *et al.*, 1990; Bjork *et al.*, 1994). Differences in GAD reactivity between the stiff-man syndrome and insulin-dependent diabetes indicate that there are differences in antigen presentation to the immune system during the development of these diseases (Bjork *et al.*, 1994). This hypothesis is supported by the observation that GAD is the only islet cell antigen recognized by islet cell antibodies in patients with the stiff-man syndrome, whereas sera from newly diagnosed insulin-dependent diabetics recognize other islet cell antigens in addition to GAD (Richter *et al.*, 1993).

Both soluble and membrane forms of GAD contribute to the activity of GAD in the brain (Nathan *et al.*, 1994). There are two isoforms of soluble GAD, a 65-kDa form (GAD-65) and a 67-kDa form (GAD-67), which are the products of two different genes and differ substantially only at their N-terminal regions (Bu *et al.*, 1992). Both proteins are expressed in the brain, but their expression in pancreatic beta cells varies among species (Petersen *et al.*, 1993; Velloso *et al.*, 1993). In neurones GAD is concentrated around synaptic vesicles, and in pancreatic beta cells it is concentrated around synaptic-like microvesicles and in the region of the Golgi complex (Reetz *et al.*, 1991). By separately expressing the cloned genes for GAD-65 and GAD-67 in Chinese hamster ovary cells and COS cells, Solimena *et al.* (1993) studied the mechanism of the subcellular targeting of GAD. They found that GAD-67 had a diffuse cytoplasmic localization, whereas GAD-65 had a punctate distribution that was mainly concentrated in the area of the Golgi complex. A chimeric protein in which the 88 N-terminal amino acid residues of GAD-67 had been replaced by the 83 N-terminal amino acid residues of GAD-65 was targeted to the Golgi complex, indicating that the

N-terminal region of GAD-65 contains a targeting signal sufficient for directing the remaining portion of the molecule, highly similar in GAD-65 and GAD-67, to the Golgi complex-associated structures (Solimena *et al.*, 1993).

In patients with the stiff-man syndrome the anti-GAD antibodies recognize GAD-65 but not GAD-67 on Western blots (Butler *et al.*, 1993; Li *et al.*, 1994). Butler *et al.* (1993) found that these antibodies recognized a conformational epitope in the C-terminal region (amino acid residues 475–585) of GAD-65 and at least one epitope in the N-terminal domain of GAD-65 (amino acid residues 1–95). Li *et al.* (1994) found that the antibodies recognized linear epitopes at 354–368 and, in one patient, 390–403 of GAD-65. Interestingly, the 390–403 region includes the binding site of the GAD cofactor, pyridoxal 5′-phosphate, suggesting that some anti-GAD antibodies may block the active site. Antibodies reactive to the membrane form of GAD have been found in the sera of patients with insulin-dependent diabetes mellitus (Nathan *et al.*, 1994), but it is unknown whether the sera of patients with the stiff-man syndrome exhibit this reactivity. Because the membrane form of GAD is presumed to have exposed extracellular domains, Nathan *et al.* (1994) have suggested that it is more likely than the soluble form of GAD to be involved in the pathogenesis of insulin-dependent diabetes and the stiff-man syndrome.

Antibodies against amphiphysin

Folli *et al.* (1993) found that patients with the stiff-man syndrome and breast cancer had serum autoantibodies directed against a 128-kDa brain antigen but did not have anti-GAD antibodies. They did not detect antibodies against this 128-kDa antigen in the sera of patients with the stiff-man syndrome without cancer or in the sera of patients with cancer without the syndrome. Grimaldi *et al.* (1993) also found antibodies against a 125/130-kDa brain protein, but not against GAD, in one patient with the stiff-man syndrome and colon cancer and in another with the syndrome and Hodgkin's lymphoma. Folli *et al.* demonstrated that this antigen was concentrated at synapses and had a highly restricted distribution outside the nervous system: it was subsequently identified as amphiphysin (De Camilli *et al.*, 1993), a recently discovered synaptic vesicle-associated protein (Lichte *et al.*, 1992). Unlike GAD, which is expressed only by GABA-secreting neurones, amphiphysin is not restricted to these neurones (Lichte *et al.*, 1992; Folli *et al.*, 1993). Although amphiphysin has not been detected in breast cancer tissue (Folli *et al.*, 1993), the stiff-man syndrome associated with cancer and anti-amphiphysin antibodies has the characteristics of an autoimmune paraneoplastic neurological disorder (see Chapter 12). The detection of anti-amphiphysin antibodies in patients with the stiff-man

syndrome is an indication to search for an occult cancer, particularly of the breast (Folli *et al.*, 1993).

Amphiphysin and GAD are similar in that they are both non-intrinsic membrane proteins that are concentrated in nerve terminals where they are associated with the cytoplasmic surface of synaptic vesicles. They are the only two known targets of CNS autoimmunity with this subcellular distribution, suggesting a link between autoimmunity directed against cytoplasmic proteins associated with synaptic vesicles and the stiff-man syndrome (De Camilli *et al.*, 1993).

Antibodies against other neuronal antigens

Some patients with the stiff-man syndrome have antibodies against GABAergic neurones that do not recognize GAD (Solimena *et al.*, 1990; Gorin *et al.*, 1990). Serum antibodies recognizing an 80-kDa neuronal antigen, but not GAD, have been detected in two patients with the stiff-man syndrome (Darnell *et al.*, 1993). Immunohistochemistry demonstrated neuronal binding identical to that reported with anti-GAD antibodies and both sera depleted GAD activity from brain extracts, suggesting that the 80-kDa antigen was either a different form of GAD or a protein that co-immunoprecipitates with GAD. Anti-GAD antibodies together with antibodies reacting with an additional neuronal antigen(s) have been found in some patients with the stiff-man syndrome (Richter *et al.*, 1993).

Immunological findings in the cerebrospinal fluid

Anti-GAD antibodies are present in the CSF of most, but not all, patients with the stiff-man syndrome and serum anti-GAD antibodies (Solimena *et al.*, 1990). The presence of oligoclonal IgG bands in the CSF but not the serum in some patients with the stiff-man syndrome indicates intrathecal antibody synthesis, but it has not been determined whether this intrathecal synthesis involves anti-GAD antibodies (Solimena *et al.*, 1988, 1990). Patients with the stiff-man syndrome, breast cancer and serum anti-amphiphysin antibodies also have anti-amphiphysin antibodies in the CSF (Folli *et al.*, 1993).

Mechanism by which the autoimmune process interferes with the function of the nervous system

The presence of anti-GAD antibodies or anti-amphiphysin antibodies in patients with the stiff-man syndrome suggests that this disorder results from

an autoimmune process directed against these synaptic vesicle-associated antigens; however, it is not known whether the antibodies themselves are pathogenic. Furthermore, the possible role of anti-GAD or anti-amphiphysin T cells in the pathogenesis of the stiff-man syndrome has not yet been examined. T cells specific for GAD play an important role in the spontaneous development of insulin-dependent diabetes in the non-obese diabetic mouse (Kaufman *et al.*, 1993; Tisch *et al.*, 1993), and patients with insulin-dependent diabetes exhibit increased proliferation of peripheral blood T cells in the presence of GAD-67 (Honeyman, Cram & Harrison, 1993).

Therapy

Diazepam, which potentiates the effect of endogenously released GABA on GABA receptors, is the most effective drug in the treatment of the stiff-man syndrome (Lorish *et al.*, 1989). Other drugs which may sometimes be beneficial include oral baclofen, clonazepam and sodium valproate (Lorish *et al.*, 1989). Patients who lose their responsiveness to diazepam as the disease progresses can benefit from the intrathecal administration of baclofen, a GABA agonist, by a programmable drug pump (Penn & Mangieri, 1993). Paraspinal muscle injection of botulinum toxin A was found to be beneficial in one patient (Davis & Jabbari, 1993). With regard to immunotherapy, plasmapheresis is beneficial in some patients with the stiff-man syndrome (Vicari *et al.*, 1989; Brashear & Phillips, 1991) but not in others (Harding *et al.*, 1989), and corticosteroid therapy also has resulted in improvement in some, but not all, patients (Piccolo *et al.*, 1988; Vicari *et al.*, 1989; Harding *et al.*, 1989). Further studies will be required to determine the place of plasmapheresis and immunosuppressant therapy in the management of patients with the stiff-man syndrome.

Conclusions

The hypothesis that the stiff-man syndrome is an autoimmune disease of the CNS is supported by the following observations: the association with HLA-DQB1*0201; the presence of oligoclonal IgG bands in the CSF; the finding of perivascular lymphocytic infiltration in the CNS; the presence of anti-GAD antibodies and the association with organ-specific autoimmune disease in a significant proportion of patients; the presence of anti-amphiphysin antibodies and association with remote malignancy in some of the other patients with this syndrome; and the beneficial effect of plasmapheresis in

some patients. However, further studies will be required to determine the role of these anti-neuronal antibodies and the role of specific T cells in the pathogenesis of the disorder, as well as to determine the place of immunotherapy in patient management. The availability of recombinant GAD and amphiphysin may allow the development of animal models to facilitate studies on the pathogenesis of the stiff-man syndrome.

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